OBSERVATIONS ON THE ACIDITY AND ALKALINITY OF THE BLOOD IN TRYPANOSOME INFECTIONS

BY

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Yakimoff[1] in his publications dealing with the changes of blood during trypanosome infection states that the alkalinity of blood decreases as the disease progresses. He used for his estimations von Limbeck's[2] method, whereby the serum alkalinity is measured against litmus. This method, however, does not indicate the true alkalinity of the serum, as the results are affected by the increase of the acidity and the carbon dioxide present in the blood.

A method which practically eliminates these errors has been described recently by Moore and Wilson,[3] who estimate the alkalinity of the ash after incineration of the blood. We were able to show, with their method, that during infections with *T. brucei* and *T. equiperdum* the acidity of the blood serum increases, whereas the alkalinity of the blood apparently remains constant.

B. Moore's and F. Wilson's technique was adopted, phenolphthaleine being used as an indicator for alkalinity, and dimethyl-amido-azo-benzol (referred to as dimethyl for brevity) for acidity. In addition titrations were made with Congo red, as this does not indicate organic acids such as amido acids.[4]

It is remarkable that in cases where both indicators were used, the acidity against Congo red was lower than against phenolphthaleine. This difference becomes more marked as the infection progresses, a fact which seems to suggest that trypanosome infection causes an increase of amido acids in the blood.

To eliminate the error which might be produced by the CO_2 in the breath, to which Moore and Wilson allude in their paper, special

precautions were taken. The mouth-part of the pipette contained KOH between two layers of cotton wool, so that all the CO_2 of the breath was absorbed by the potassium hydroxide.

The blood was collected in small test tubes and left standing over night in an ice chest; then the serum was separated from the clot and used for the estimation of acidity. For the estimation of the alkalinity, the blood was collected in a platinum crucible and incinerated. All the glass vessels used in this work were immersed in strong hydrochloric acid for three days, and then in distilled water for the same length of time, so as to avoid the error which might be produced by the alkalinity of the glass.

Experiment No. 303.—Rabbit, male, inoculated on 10th February, 1908, with *T. equiperdum*. On 12th February the acidity was 0·03015* (phenolphthaleine); on 24th February the acidity had increased to 0·03255 (phenolphthaleine), 0·03195 (congo red), the alkalinity was 0·02895. The animal was frequently examined and kept under observation until 7th April, when the acidity had reached 0·03495 (phenolphthaleine), 0·03345 (congo red), and the alkalinity 0·03015.

Experiment No. 305.—Rabbit, female, inoculated on 10th February, 1908, with *T. equiperdum*. On 24th February the acidity was 0·03285 (phenolphthaleine), 0·03225 (congo red), and the alkalinity 0·02865. On 7th April the acidity had reached 0·02865 (phenolphthaleine), 0·03405 (congo red), and the alkalinity 0·02925. It is interesting to note that on 31st March the acidities against congo red and phenolphthaleine were the same, 0·03465.

Experiment No. 307.—Guinea-pig, female, inoculated on 24th February, 1908, with *T. brucei*. The first alkalinity and acidity estimations were made on 26th February, when the acidities were found to be 0·03105 (phenolphthaleine), 0·03075 (congo red), and the alkalinity 0·03045. On 11th March the animal was found to be swarming with parasites, and the acidity estimation on 14th March gave 0·03435 (phenolphthaleine), 0·0327 (congo red), and the alkalinity 0·03000. The animal died on 18th March of typical trypanosomiasis. The acidity estimation made twenty minutes after death gave 0·03615 (phenolphthaleine), and 0·03480 (congo red).

Experiment No. 309.—Guinea-pig, female, inoculated on 24th February, 1908, with *T. brucei*. The first acidity estimation was made on 26th February, and found to be 0·03195 (phenolphthaleine), 0·03175 (congo red), and the alkalinity 0·03030. The animal showed trypanosomes on the same day. On 7th April the acidity had reached 0·03465 (phenolphthaleine), 0·03450 (congo red), and the alkalinity 0·02925: the animal was then heavily infected.

Experiment No. 310.—Guinea-pig, female, inoculated on 24th February, 1908, with *T. brucei*. On 26th February the acidity was 0·03225 (phenolphthaleine), 0·03210 (congo red), and the alkalinity 0·02895. On 8th March the acidity was 0·03405 (phenolphthaleine), 0·03300 (congo red). A fall in the acidity was noticed on 14th March. The acidity reached 0·03195 (phenolphthaleine), and the same also for congo red. Afterwards the acidity started slowly to increase and became on March 23rd 0·03510 (phenolphthaleine), 0·03375 (congo red), and the alkalinity 0·02985. On 7th April the acidity had reached 0·03585 (phenolphthaleine), 0·03495 (congo red), and the alkalinity 0·03045.

* Expressed in fractions of Normal.

Experiment No. 311.—Guinea-pig, male, inoculated on 24th February, 1908, with *T. brucei*. On 24th February the acidity was found to be 0·03245 (phenolphthaleine), 0·03225 (congo red), and the alkalinity 0·03000. The last estimation was made on 17th March, and the animal died on 19th March. The animal was then slightly infected, and the acidity found to be 0·03300 (phenolphthaleine), 0·03255 (congo red), and the alkalinity 0·03000. The cause of the death of this animal was pneumonia.

Experiment No. 314.—Rabbit, female, inoculated on 14th March, 1908, with *T. brucei*. On the day of inoculation the acidity was found to be 0·03075 (phenolphthaleine), 0·03030 (congo red), and the alkalinity 0·02850. On 23rd March the acidity was found to be 0·03300 (phenolphthaleine), 0·03195 (congo red), and the alkalinity 0·03060. On the same day, three young ones were born which soon died. The acidity on 26th March had dropped down to 0·03240 (phenolphthaleine), 0·03225 (congo red), and the alkalinity 0·02850. From this day the acidity increased slowly, and on 7th April was 0·03345 (phenolphthaleine), 0·03285 (congo red), and the alkalinity 0·03015. The animal died on 9th February. This animal was positive all the time, starting from 17th March.

Experiment No. 351.—Rabbit, female, inoculated on 14th March, 1908, with *T. brucei*. The acidity was then 0·03120 (phenolphthaleine), 0·03075 (congo red), and the alkalinity 0·02940. On April 7th the acidity had reached 0·03375 (phenolphthaleine), 0·03315 (congo red), and the alkalinity 0·02910.

Experiment No. 316.—Rabbit, male, inoculated on 14th March, 1908, with *T. brucei*, showing an acidity of 0·03180 (phenolphthaleine), and 0·03075 (congo red), and an alkalinity of 0·02880. When examined on 31st March, the acidity had reached 0·03375 (phenolphthaleine) 0·03210 (congo red), and the alkalinity 0·03000.

Experiment No. 317.—Guinea-pig, female, inoculated on 14th March, 1908, with *T. brucei*. The acidity was then 0·03150 (phenolphthaleine), 0·03105 (congo red), and the alkalinity 0·02895. The animal died on 8th April of typical trypanosomiasis On 7th April the acidity had reached 0·03585 (phenolpbthaleine), and 0·03360 (congo red), when the alkalinity was 0·02850.

I should like especially to draw attention to this experiment, in which both the increase of the total acidity and also that of the amido acids is very marked.

The following table gives in full the changes in acidity and alkalinity during an experimental infection:—

Experiment No. 304.—Rabbit, male, inoculated 10th February, 1908, with *T. equiperdum*.

Date	Acidity to phenolphthaleine	Acidity to congo red	Alkalinity to dimethyl
12/2/08	0·03135	—	—
14/2/08	0·03195	—	—
18/2/08	0·03225	—	0·02895
20/2/08	0·03285	0·03195	0·02880
24/2/08	0·03240	0·03210	0·02880
27/2/08	0·03240	0·03225	—
2/3/08	0·03255	0·03210	0·02895
4/3/08	0·03300	0·03210	0·02865
8/3/08	0·03255	0·03225	—
10/3/08	0·03285	0·03210	0·02895
14/3/08	0·03255	0·03210	0·02865
17/3/08	0·03255	0·03255	0·02880
19/3/08	0·03285	0·03225	0·02910
23/3/08	0·03315	0·03225	0·02985

N.B.—Trypanosomes examinations were made daily. The animal died on 25/3/08, when the *post-mortem* showed a more or less marked trypanosomiasis.

The following table shows the acidity and alkalinity of normal rabbit serum :—

Sex	Acidity to phenolphthaleine	Acidity to congo red	Alkalinity to dimethyl
F.	0·03175	—	—
M.	0·03225	—	—
F.	0·03075	—	—
F.	0·03180	—	—
F.	0·03075	—	—
F.	0·03120	—	—
M.	0·03090	—	—
F.	0·03120	—	—
F.	0·03015	—	—
F.	0·03175	—	—
F.	0·03075	—	—
F.	0·03120	—	—
M.	0·03015	—	—
F.	0·03150	—	—
F.	0·03075	0·03075	0·02895
M.	0·03175	0·03105	0·02850
F.	0·03075	0·03120	0·02895
F.	0·03195	0·03060	0·02955
F.	0·03175	0·03030	0·02865
F.	0·03050	0·03050	0·03045
F.	0·03120	0·03050	0·03000
M.	0·03030	0·03015	0·02880
F.	0·03075	0·03030	0·02850
M.	0·03180	0·03075	0·02880
F.	0·03120	0·03075	0·02940
Average	0·03115	Average 0·03064	Average 0·03025

Conclusions.

I. It is evident, that in experimental trypanosomiasis infection (*T. brucei* and *T. equiperdum*), the acidity of the blood increases.

II. The increase of the acidity is probably due to the production of amido acids through, or by the trypanosomes, i.e., the acids might be either secreted by the parasites or produced by the action of the parasites on the proteins of the blood serum. In the latter case, the amido acids would be broken up through hydrolysis from the proteins into simpler polipeptids.

III. It is possible that the increase of acidity might be of assistance in the diagnosis of a typical case of trypanosomiasis, where the parasites have disappeared for some length of time from the blood circulation.

IV. These experiments suggest that in trypanosome treatment effort should be made to neutralise the increased acidity of the blood, as this might prove of additional assistance in making the blood a less favourable medium for their development.

LITERATURE

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